

# ADVANCED HEALTHCARE MATERIALS

## Supporting Information

for *Adv. Healthcare Mater.*, DOI: 10.1002/adhm.202001847

Development of a biomimetic hydrogel based on pre-differentiated mesenchymal stem cells-derived ECM for cartilage tissue engineering

*Cristina Antich*<sup>1,2,3,4</sup>, *Gema Jiménez*<sup>2,3,4,5</sup>, *Juan de Vicente*<sup>4,6</sup>, *Elena López*<sup>2,3,4,5</sup>, *Carlos Chocarro*<sup>1,2,3,4</sup>, *Carmen Griñán*<sup>1,2,3,4</sup>, *Esmeralda Carrillo*<sup>1,2,3,4</sup>, *Elvira Montañez*<sup>7,8</sup>, *Juan A. Marchal*<sup>1,2,3,4,\*</sup>

# Supporting Information

Development of a biomimetic hydrogel based on pre-differentiated mesenchymal stem cells-derived ECM for cartilage tissue engineering

*Cristina Antich*<sup>1,2,3,4</sup>, *Gema Jiménez*<sup>2,3,4,5</sup>, *Juan de Vicente*<sup>4,6</sup>, *Elena López-Ruíz*<sup>2,3,4,5</sup>,  
*Carlos Chocarro-Wrona*<sup>1,2,3,4</sup>, *Carmen Griñán-Lisón*<sup>1,2,3,4</sup>, *Esmeralda Carrillo*<sup>1,2,3,4</sup>,  
*Elvira Montañez*<sup>7,8</sup>, *Juan A. Marchal*<sup>1,2,3,4,\*</sup>

\* Corresponding author: Juan A. Marchal, M.D., Ph.D. Prof, Department of Human Anatomy and Embryology, School of Medicine, University of Granada, E-18016 Granada, Spain, Ph: 958241000 Ext 20080, [jmarchal@ugr.es](mailto:jmarchal@ugr.es)

## Methods

### *Isolation and culture of chondrocytes*

Articular cartilage was obtained from patients with osteoarthritis during joint replacement surgery after informed consent from all patients and approval from the Ethics Committee of Clinical University Hospital of Málaga, Spain. Sample was isolated from the femoral side, selecting the non-overload compartment: lateral condyle in varus knees and medial condyle in the valgus cases. None of the patients had a history of inflammatory arthritis or crystal-induced

arthritis and only cartilage that macroscopically looked relatively normal was used for this study.

#### *Quantitative biochemistry assays*

GAGs content was estimated via quantifying the amount of sulphated GAGs by Dimethyl Methylene Blue (DMMB) colorimetric assay. With that aim, samples were digested in 1 ml of papain solution (125 mg/ml papain in 0.1 M sodium phosphate with 5 mM Na<sub>2</sub>-EDTA and 5 mM cysteine-HCl at pH 6.5) for 16 h at 60°C. The resulting extract was mixed with DMMB solution to bind GAGs. The content was calculated based on a standard curve of sulphate chondroitin from shark cartilage (Sigma) at 570 nm on a microplate spectrophotometer.

Total collagen content was measured by picosirius red staining. For this, it was firstly solubilized by the incubation of the matrix with pepsin in acetic acid 0.5 M (2 mg/ml) and stained with 1 mL of Sirius red dye for 30 min at room. Then, the stain was dissolved using 0.5 N Sodium Hydroxide (0.5 N NaOH) and the absorbance was measured at 560 nm using a Microplate Reader (Berthold technologies, USA). Collagen type I (rat tail) was used as standard for the biochemical assays.

Collagen type II content was determined using a commercially available Collagen type II ELISA kit (Chondrex). For this, samples were digested by pepsin (1 mg/ml) in 0.5 N acetic acid for 48h at 4°C followed by adding 1 mg/ml pancreatic elastase solution at 4°C for 24 h. Finally, these were neutralized with 1 M Tris base. Insoluble material was removed by centrifugation at

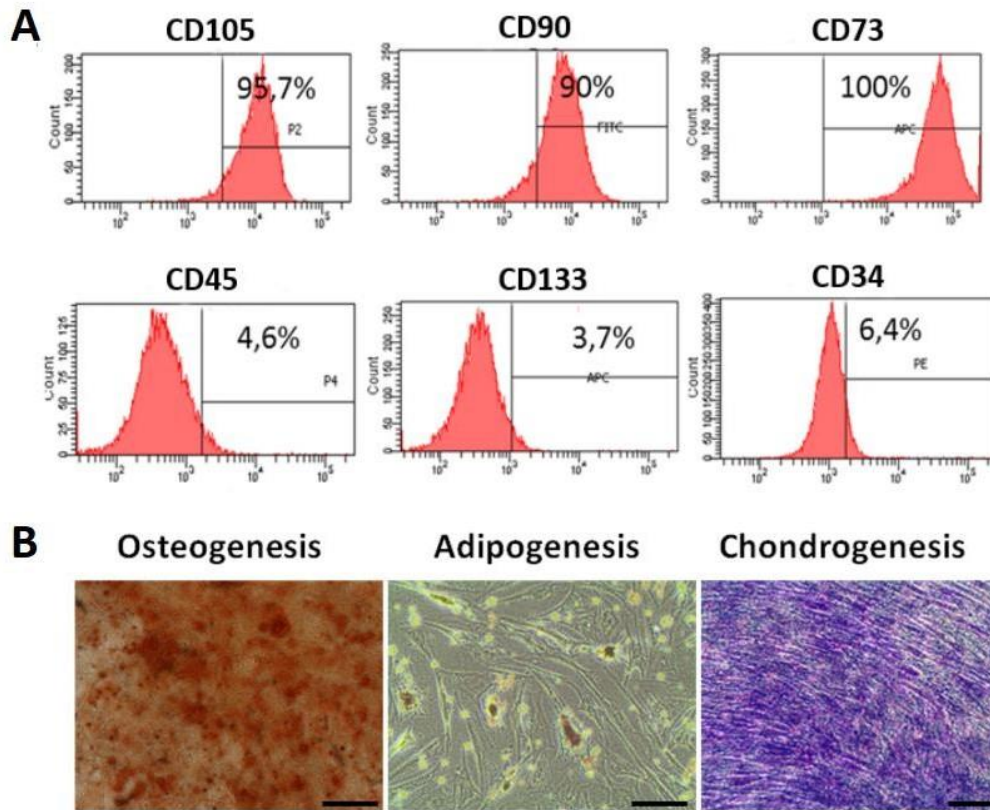
10,000 rpm at RT for 5 min, and the supernatant was collected for assay. Quantitative analysis was performed according to manufacturer's instruction and measured on micro-plate spectrophotometer at 490 nm.

DNA content was quantified by fluorometric assay using DAPI staining. For this, samples were digested in papain solution for 16 h at 60°C. The resulting extract was stained with DAPI. The fluorescence intensity was measured in fluorescence spectrophotometer (excitation wavelength: 360 nm, emission wavelength: 450 nm). Calf thymus DNA was used to create a standard curve for quantifying the DNA in samples.

#### *Analysis by Mass Spectrometry (MS)*

To more fully characterize the protein content of mdECM, tandem mass spectroscopy (MS/MS) was performed. Samples were resuspended in 50 mM  $\text{NH}_4\text{HCO}_3$  (pH 8.5) and a small part (1/20) was digested. Proteins were disaggregated by ultrasonic bath and mixing. Briefly, proteins were reduced (DTT 21 mM; 1h, 56°C) and alkylated (iodoacetamide 55 mM; at RT for 30 min, in the dark). Afterwards, proteins were digested overnight with trypsin (Sequence grade modified porcine Trypsin, Promega; pH 8, 37°C), the next day a double digestion was performed with the same enzyme. The resulting peptide mixtures were cleaned-up with a C18 tip (PolyLC Inc.) as per manufacturer's protocol. Finally, the cleaned-up peptide solutions were dried-down and stored at -20 °C until the LC/MS analyses. The dried-down peptide mixtures were analyzed in a nanoAcquity liquid chromatographer (Waters) coupled to a LTQ-Orbitrap Velos (Thermo Scientific) mass spectrometer. Peptides were trapped on a Symmetry

C18™ trap column (5µm 180µm × 20mm; Waters), and were separated using a C18 reverse phase capillary column (ACQUITY UPLC M-Class Peptide BEH column; 130Å, 1.7µm, 75 µm × 250 mm, Waters). The gradient used for the elution of the peptides was 1 to 40 % B in 30 minutes, followed by gradient from 40% to 60% in 5min (A: 0.1% FA; B: 100% ACN, 0.1%FA), with a 250 nL/min flow rate. Eluted peptides were subjected to electrospray ionization in an emitter needle (PicoTip™, New Objective) with an applied voltage of 2000V. Peptide masses ( $m/z$  300-1700) were analyzed in data dependent mode where a full Scan MS was acquired in the Orbitrap with a resolution of 60,000 FWHM at 400m/z. Up to the 15th most abundant peptides (minimum intensity of 500 counts) were selected from each MS scan and then fragmented in the linear ion trap using CID (38% normalized collision energy) with helium as the collision gas. The scan time settings were: Full MS: 250 ms (1 microscan) and MSn: 120 ms. The generated .raw data file was collected with *Thermo Xcalibur* (v.2.2). The raw files obtained in the mass spectrometry analyses were used to search against a modified version of the public database SwissProt human. A small database containing laboratory contaminant proteins was also added. Database search was performed with Sequest HT search engine using Thermo Proteome Discover (v.1.4.1.14). The search results were visualized in Proteome Discoverer (v.1.4.1.14) and exported to Excel as lists of identified proteins. The results have been filtered so only proteins identified with at least 2 high confidence peptides (FDR > 1%) are included in the list.



**Figure S1.** Phenotypic characterization and differentiation potential of MSCs. **(A)** FACS characterization of MSCs showed a positive expression of surface markers CD105, CD90, CD73 and negative or low expression for CD45, CD133 and CD34. **(B)** The differentiation potential of MSCs obtained from lipoaspirate towards osteogenic, adipogenic and chondrogenic lineage was confirmed by alizarin red S, oil red O, and toluidine blue staining, respectively. Scale bar: 100  $\mu$ m.